Application. No.: 09/836,14 Applicant: Cravatt, et. al.

Filed: April 16, 2001

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PATENT
Attorney Docket No.: SCRIP1210-3

On page 43, before paragraph 147, insert a title of the Example: PREPARATION OF FP-PEG-BIOTIN"

## IN THE CLAIMS

Please add the following new claims.

--14. A method for screening for molecules having an affinity for an active target protein in a complex mixture of proteins from a biological source, employing a combinatorial chemical library of activity based probes comprising a plurality of members of the formula

$$R*(F-L)-X$$

wherein:

X is a ligand having the same chemical structure for each of said members of said library; L is a bond or alkylene or an alkyleneoxy chain linking group of from 1 to 6 alkyleneoxy groups, wherein said alkyleneoxy groups are of from 2 to 3 carbon atoms, which is the same in each of the members of said library,

F is a sulfonyl group that is the same in each member of said library; and R is a group of less than 1kDal, that is different in each of the members of the library; the \* denotes that R is a part of F or L; said method comprising:

- (1) combining a first portion of said complex mixture with said combinatorial chemical library under conditions for reaction of said sulfonyl group with active proteins in said complex mixture to form conjugates;
- (2) combining a second portion of said complex mixture, that has been inactivated, with said combinatorial library under the same reaction conditions as in (1)
- (3) isolating conjugates from said first and second portions of said complex mixture; and
- (4) comparing conjugates formed from said first portion of said complex mixture with conjugates formed from said second portion of said complex mixture to determine the degree of activity of the total target protein as compared to active target protein.



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- 15. A method according to claim 14, wherein each of said members of said combinatorial chemical library of activity based probes is isotopically labeled, said method including the additional step of analyzing said conjugates for the composition of proteins bound to members of said library.
- 16. A method according to claim 14, wherein said members of said combinatorial chemical library of activity based probes have differing on-rates with respect to said active proteins.
- 17. A method according to claim 14, wherein X is biotin, deiminobiotin, dethiobiotin, a vicinal diol, digoxigenin, maltose, oligohistidine, glutathione, 2,4-dinitrobenzene, phenylarsenate, ssDNA, ds DNA, a peptide, metal chelate, saccharide, rhodamine, fluorescein, or a hapten.
- 18. A method according to claim 14, wherein X is biotin.
- 19. A method according to claim 14, wherein X is rhodamine.
- 20. A method according to claim 14, wherein R is alkyl, heterocyclic, aryl, substituted aryl, amino acid, peptidyl, oligonucleotide, or a carbohydrate group.
- 21. A method according to claim 14, wherein F is sulfonate, sulfate, sulfinate, or sulfamate.
- 22. A method according to claim 21, wherein F is sulfonate.
- 23. A method according to claim 14, wherein said second portion of said complex proteomic mixture has been inactivated by heating.